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Short Report

Disposable Collection Kit for Rapid and Reliable Collection of Saliva

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Objectives: To describe and evaluate disposable saliva collection kit for rapid, reliable, and reproducible collection of saliva samples.

Methods: The saliva collection kit comprised of a saliva absorbent swab and an extractor unit was used to retrieve whole saliva samples from 10 subjects. The accuracy and precision of the extracted volumes (3, 10, and 30 μl) were compared to similar volumes drawn from control samples obtained by passive drool. Additionally, the impact of kit collection method on subsequent immunoassay results was verified by assessing salivary cortisol levels in the samples and comparing them to controls.

Results: The recovered volumes for the whole saliva samples were 3.85 ± 0.28, 10.79 ± 0.95, and 31.18 ± 1.72 μl, respectively ($CV = 8.76\%$) and 2.91 ± 0.19, 9.75 ± 0.43, and 29.64 ± 0.91 μl, respectively, ($CV = 6.36\%$) for the controls. There was a close correspondence between the salivary cortisol levels from the saliva samples obtained by the collection kit and the controls ($R^2 > 0.96$).

Conclusions: The disposable saliva collection kit allows accurate and repeatable collection of fixed amounts of whole saliva and does not interfere with subsequent measurements of salivary cortisol. The simple collection process, lack of elaborate specimen recovery steps, and the short turnaround time (<3 min) should render the kit attractive to test subjects and researchers alike. Am. J. Hum. Biol. 27:720–723, 2015.

The profiles of biological markers in blood and urine are commonly used as indicators of the health–disease continuum. However, the relative intrusiveness, handling complexity, and processing costs of blood and urine samples render saliva an attractive alternate (Mandel, 1993; Koh and Koh, 2007; Zhang et al., 2012). Saliva is readily and repeatedly obtainable, inexpensive to collect, carries a very low risk of pathogen transmission and is simpler to handle, transport, and process (Granger et al., 2007, 2012). Unlike blood and urine, the noninvasive nature of saliva collection can eliminate anxiety, discomfort or embarrassment, and make saliva particularly suitable for field studies using biomarkers to investigate the psychobiological mechanisms of stress-related disorders (Kirschbaum and Hellhammer, 1994; Hellhammer et al., 2009).

An important first step for accurate analysis of the salivary constituents is the ability to conveniently, reliably and repeatedly collect adequate volumes of saliva samples. Generally, saliva is collected either by the passive drool technique or by the absorbent device technique. In the passive drool technique, nonstimulated whole saliva pooling at the floor of the mouth is gathered in a collection tube. However, the collection of saliva by drooling is a messy task and can discourage individuals from providing repeated samples. Incorporated foam or particulate matter can interfere with subsequent assays. The alternate absorbent device approach utilizes a spongy material that is placed in the mouth for 2–3 min and allowed to soak with saliva. Once saturated, the material is removed and the saliva extracted by centrifugation or compression (Granger et al., 2007, 2012). The absorbent material approach is popular because it is simple and convenient and allows individuals to self-collect with minimal instruction and minimal embarrassment. Irrespective of the approach used, the sample integrity, measurement reliability, and validity can be easily influenced by variations in the flow rates or volume of the saliva collected. The absorbent device approach is particularly sensitive to the placement of absorbent device and may end up collecting localized saliva rather than whole saliva, thereby affecting results for many analytes. The multistep saliva extraction methods, ranging from centrifugation to aliquoting, can be tedious and time-consuming when applied to large batches and not easily employed in newer point-of-use analytical systems that are replacing distant laboratory assays (Yamaguchi and Shetty, 2012; Kaushik et al., 2014). Additionally, the commonly used cotton-based absorbent materials can distort quantitative estimates of the salivary analytes through nonspecific binding, cross-linking or by filtering out constituent biomolecules (Shirtcliff et al., 2001; Granger et al. 2007).

To simplify the collection and recovery of saliva samples and increase the accuracy and reliability of subsequent assays, we have designed a disposable saliva collection kit that is compatible with point-of-care analytical systems. Here, we report on the ability of the kit to facilitate rapid and repeatable collection of fixed amounts of whole saliva. As proof of concept, we utilize salivary cortisol, a

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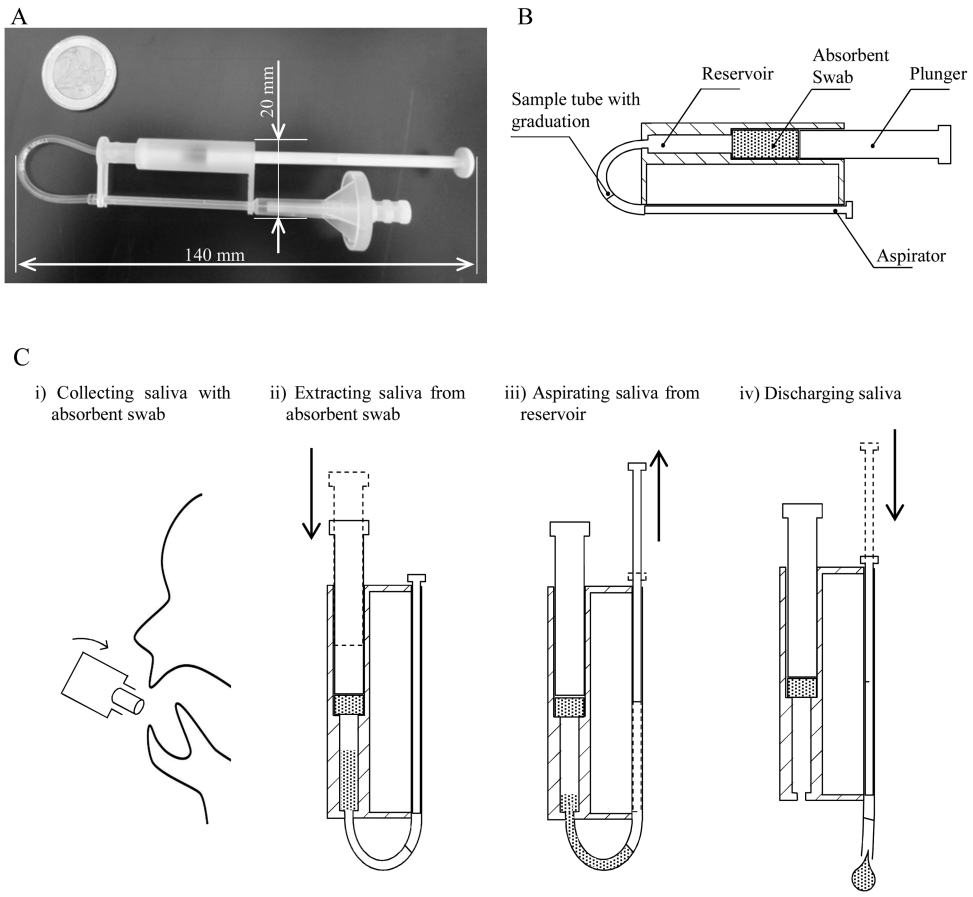

Fig. 1. Disposable saliva collection kit with component elements: main body with a sleeve for compression and a sleeve for determining volume, a plunger, a aspirator, an absorbent swab (D4924B, Filtrona Porous Technologies Co., VA), and a sample tube (0.79 mm of inner diameter and 2.38 mm of outer diameter, Saint-Gobain K.K. Co., Ltd., Japan) with corresponding graduations (A) external view, (B) schematics of assembly, and (C) operational steps.

commonly used biomarker of the stress response, to verify the impact of the collection method on subsequent immunoassay results.

MATERIALS AND METHODS

Saliva collection kit

The disposable saliva collection kit is comprised of a saliva absorbent swab and an extractor unit (Fig. 1A, B). The absorbent swab (20 mm length × 3.5 mm diameter) consists of a sponge-like braided core of polypropylene resin foam (fiber density of 0.09 g/cc) contained within a polyethylene sleeve. The saliva extractor consists of a polypropylene barrel for receiving the absorbent swab and a corresponding plunger for squeezing out the contained saliva. The distal end of the barrel opens into a narrower reservoir that stores the expressed saliva. The reservoir empties into a sample tube whose inner surface is treated with a surfactant using sucrose esters of fatty acids (DK Ester S-L18A, Dai-Ichi Kogyo Seiyaku Co., Ltd., Japan). Printed graduation marks on the sample tube allow saliva volumes to be easily verified. A contiguous aspirator permits controlled withdrawal of saliva from the reservoir into the sample tube.

To obtain a saliva specimen nonstimulated whole saliva is allowed to pool at the floor of the mouth for approximately 1 min. The subject is instructed to place the absorbent swab under the tongue for another minute (Fig. 1C-i). The constant dimensions and microstructure of the resin foam ensures that the absorbent swab saturates with consistent quantities (≈100 μl) of saliva within a minute and is independent of the salivary flow rates. The saturated swab is retrieved by expressing swab directly into the barrel of saliva extractor. The plunger is inserted and pushed down to express the contained saliva into the reservoir (Fig. 1C-ii). Next, the aspirator is activated to draw saliva held within the reservoir into the sample tube until the level reaches the graduation mark corresponding to the desired volume (Fig. 1C-iii). The constant inner diameter of the sample tube (0.79 mm) ensures that the aspirate volume is replicable and precise. Finally, the sample tube is disconnected from the reservoir and the aspirate plunger pushed to discharge a precise amount of saliva for assay purposes (Fig. 1C-iv).

Verification of salvia volume recovery

The saliva collection kit was used to obtain whole saliva samples from 10 consenting, healthy adult subjects (22.6 years ± 1.3 year) using procedures approved by the Institutional Review Board. Concomitantly, we also used the collection kit to collect corresponding volumes ($n = 10$)

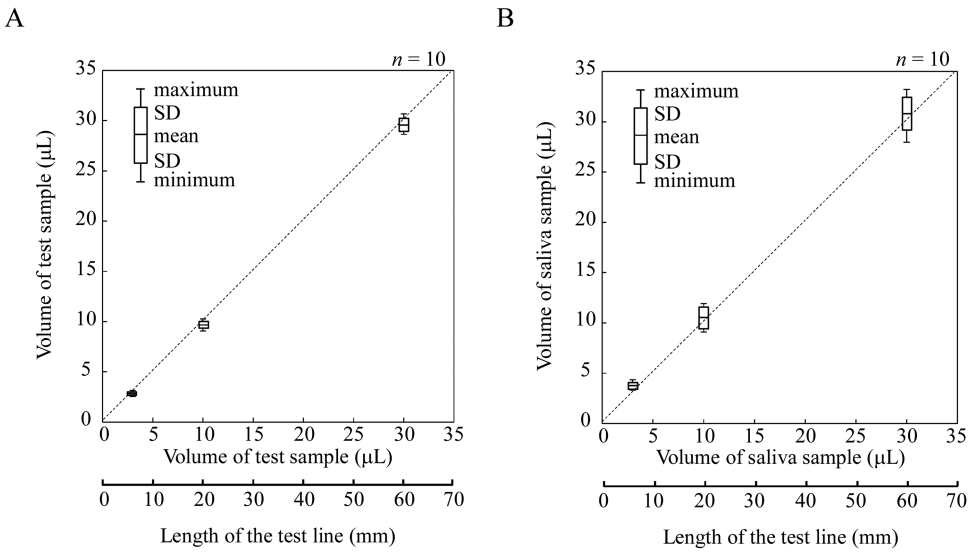

Fig. 2. Concordance of volumes collected by kit from whole saliva as well as test sample. (A) test samples (cortisol standards), (B) whole saliva samples.

from standard cortisol solution included in a commercialized ELISA (enzyme-linked immune sorbent assay) kit (control sample). The volumes of collected samples (saliva and cortisol standard) were verified by weighing recovered samples on an electronic balance (10 μg resolution, GR-202, A&D Co., Ltd, Japan) and then converting measured weights into corresponding volumes.

Impact on measured levels of salivary cortisol

To determine the impact of absorbent swab on the measurement of salivary cortisol (sCortisol) levels, we compared calibration curves for cortisol concentration obtained from whole saliva samples from the saliva collection kit and the micropipetting approach.

A passive drool saliva sample (≈ 3 ml) was collected individually from a subset of five subjects. Each subject provided the sample by allowing saliva to pool at the bottom of the mouth and then expectorating the saliva into a commercially available polypropylene cup. Next, the absorbent swab from saliva collection kit was allowed to saturate with the saliva sample and then precise amounts of saliva (25 μl) expressed by the plunger were collected for subsequent assay. Each harvested saliva sample was treated differently as follows: (a) native saliva only; (b) centrifugation only (1500*g* for 15 min); (c) freeze-thawing only (frozen to −80°C and then thawed at 4°C); and (d) freeze-thaw followed by centrifugation. Subsequently, each saliva sample was divided further into five aliquots ($n = 5$).

The native saliva obtained by the micropipetting approach was pretreated following the standard processing method for a cortisol ELISA kit and used as a control for establishing concordance. Briefly, a sample was pipetted from the remainder of the passive drool saliva, frozen to −80°C and then thawed at 4°C in a refrigerator and centrifuged at 1500*g* for 15 min. A micropipette was then used to remove a fixed aliquot of the saliva (25 μl) for subsequent analysis.

Finally, the sCortisol levels from the individual aliquots (both saliva collection kit and micropipetting) were measured by using a cortisol ELISA kit (Salimetrics LLC, State College, PA) and a plate reader (ARVO MX; Perkin Elmer Life Science, Boston, MA).

Data analysis

All analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc, Chicago, IL). Linear regression was used to estimate the linear correspondence between the saliva collection kit and the conventional passive drool method. Measurement precision was quantified with the coefficient of variation (CV).

RESULTS

Accuracy and precision of the saliva collection kit was established by comparing incremental volumes collected by the kit (3, 10, and 30 μl) from both the cortisol standards and the whole saliva samples. For the cortisol standard, the measured volumes were 2.91 ± 0.19, 9.75 ± 0.43, and 29.64 ± 0.91 μl respectively, with a *CV* of 6.36% (Fig. 2A). For the whole saliva samples, the measured volumes were 3.85 ± 0.28, 10.79 ± 0.95, and 31.18 ± 1.72 μl, respectively, with a *CV* of 8.76% (Fig. 2B).

The regression analysis revealed a close correspondence between the sCortisol levels measured from the saliva samples obtained by the collection kit and by the passive drool method. The coefficient of linearity ($R^2 > 0.96$) was consistently high for all four pretreatment conditions (0.97, 0.96, 0.98, and 0.97, respectively) and the *CV*s ranged from 5.01 to 9.56 for the four pretreatment conditions (7.86, 9.56, 6.63, and 5.01%, respectively).

DISCUSSION

Our results indicate that the new saliva collection kit allows accurate and repeatable collection of fixed amounts of whole saliva and does not interfere with subsequent measurements of salivary cortisol. In our validation testing using both whole saliva samples and control samples,

the volumes dispensed by the kit were very reproducible and closely approximated the graduation marks on the sample tube. Unlike passive drool techniques that can be embarrassing to the subjects, the saliva samples dispensed by the collection kit were easily collected and devoid of any particulates or air bubbles. With the exception of subjects with dry mouth and greatly diminished saliva flow rates, the collection system is capable of saliva volumes adequate for most assays. Furthermore, the nonreactive absorbent swab did not distort quantitative estimates of the salivary analytes, a concern highlighted by Shirtcliff et al. (2001) with regard to conventional cotton absorbent pads. Using salivary cortisol as an example, we verified that immunoassay results from saliva samples collected by our kit corresponded closely to sCortisol levels from samples collected by the conventional passive drool technique, even after various pretreatment steps.

The reproducible collection, seamless recovery of samples, and accurate dispensation of saliva volumes bodes well for the reliability of subsequent assays. A streamlined collection process, lack of any elaborate specimen recovery steps, and the short turnaround time (<3 min) for retrieving the samples should render the kit attractive to test subjects and researchers alike. The immediate availability of accurate amounts of saliva samples is particularly suitable for point-of-care analysis of disease biomarkers through emerging biosensor technologies (Yamaguchi and Shetty, 2012). The simple, inexpensive and user-centric nature of the disposable collection kit should facilitate saliva sample collection in naturalistic settings for large population studies or studies with a repeated measures design.

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